REMARKS

Claims 11-27 are pending. New claim 11 corresponds to old claim 1, amended for technical clarity to include electronic input and output signals. Support for new claim 12 is found in old claim 1 and at page 4, first and second paragraphs of the detailed description. Support for new claim 13 is found in old claim 1 and at page 9, third full paragraph of the specification. Support for new claim 14 is found at page 9, third full paragraph of the specification. Support for new claim 14 is found at page 7, fourth paragraph. Support for new claims 16-23 is found in old claims 2-9. Support for new claims 24-26 is found at page 4, past paragraph and page 91, second paragraph, among other places. Support for new claim 27 is found in old claim 10.

Claims 1-9 are rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 1-10 are rejected under 35 U.S.C. § 112, second paragraph for being indefinite. Claim 1 is rejected under 35 U.S.C. § 102(a) as being anticipated by Livache et al., (1998) *Anal. Biochem.*, 255:188-194.

✓As a preliminary matter, Applicants note that the drawings are objected to. It is the applicants intention to formally correct the defects noted on PTO-948. Applicants respectfully request the rejection be held in abeyance until otherwise allowable subject matter is found.

Compliance with 37 C.F.R. §§ 1.821-1.825

Attached hereto is an Amendment in response to the Office Communication and Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disc containing the above named sequence, SEQUENCE ID NUMBERS 1-7 in computer readable form, and a paper copy of the sequence information. The computer readable sequence listing was prepared through use of the software program "PatentIn" provided by the PTO. The information contained in the computer readable disc is identical to that of the paper copy.

This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. § 1.821-1.825.

Claim Objections

Claim 4 is objected to because of the use of the abbreviation "JTFT". Claim 4 has been amended to incorporate a phrase that defines the abbreviation "JTFT". Accordingly, Applicants request withdrawal of the objection.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-9 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner indicates that the specification is not enabling for the use of signal processing methods for the detection of target analytes, other than nucleic acids.

It is well settled law that the specification must enable the scope of the claimed invention, but that the specification need not provide a specific description for each and every embodiment covered by the claimed invention. *See, e.g., Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1115 (Fed. Cir. 1991). That the claimed invention covers a wide variety of signal processing methods in accordance with the claimed invention is not relevant to the issue of enablement. Rather, the test of enablement requires that the specification, in light of the prior art, provides ample guidance to one of skill in the art, to apply the signal processing methods of the invention for the detection of target analytes. Applicants submit that the specification provides ample guidance for applying signal processing methods to the electronic detection of target analytes, in light of the prior art and the level of skill in the art. Furthermore, the specification provides specific examples of processing output signals for the electronic detection of nucleic acids. This alone is sufficient teaching to enable the scope of independent claims 1 and 10.

Summary of the Invention

The present invention is directed to the use of signal processing methods for the electrochemical detection of biomolecules. In general, the observed signal is a combination of signal from the biomolecule, e.g., target analyte, and signal from the background, e.g., noise. Methods for the electrochemical detection of biomolecules involve looking for a

response in a "biosensor", such as an electrode, to an input signal. For example, a voltage may be applied to an electrode and a change in the observed or output signal measured.

Techniques that can be used to increase the signal, decrease the noise, or make it easier to detect the signal over background noise are the object of the present invention. Specifically, the present invention applies methods of processing output signals in electrical systems to biological systems.

A number of assays rely on electronic signals for the detection of biomolecules. Of particular interest are biosensors. Listed below are examples of some of the patents and publications describing electronic detection of target analytes:

1. Meade et al., (U.S. Patent No. 5,824,473, Nucleic Acid Mediated Electron Transfer; reference 41 on the IDS filed July 5, 2000) which claims methods of detecting target sequences using initiation signals and detecting electron transfer.

2. Meade et al., (U.S. Patent No. 5,952,172, Nucleic Acid Mediated Electron Transfer; reference 55 on the IDS filed July 5, 2000), which claims methods for detecting the presence of target sequences using AC input signals.

3. Kayyem and O'Connor (U.S.S.N. Serial No. 08/911, 589, AC Detection of Nucleic Acid; cited under M.P.E.P. §2001.06(b) in the IDS filed July 5, 2000) describe methods for detecting the presence of a target sequence in a nucleic acid sample. The methods comprise applying a first input signal comprising an AC component and a non-zero DC component to a hybridization complex comprising at least a target sequence and a first probe single stranded nucleic acid. The hybridization complex is covalently attached to a first electron transfer moiety comprising an electrode, and a second electron transfer moiety. The presence of the hybridization complex is detected by receiving an output signal characteristic of electron transfer through said hybridization complex.

4. Mikkelsen et al., (U.S. Patent No. 5,312,527; reference 21 on the IDS filed July 5, 2000) describe a voltammetric sequence-selective sensor for target polynucleotide sequences. The detection electrode is an amperometric electrode that monitors local changes in a physical property that occurs at the surface of the sensor, and converts this physical property into a measurable electronic signal.

5. Millan, et al., ((1998) Anal. Chem., 66:2943-2948; reference 129 on the IDS filed July 5, 2000) describe the development of a sequence selective biosensor for DNA using carbon paste electrodes.

6. Ribi, et al., (U.S. Patent No. 5,571,568) describe bioelectronic sensor employing a thin surfactant polymeric electrically conducting layer to which may be bound members of specific binding pairs. Binding of an analyte or a reagent to the specific binding pair member layer changes the electrical, optical, or structural properties of the layer, allowing for detection of the analyte.

7. Thorp, et al, (U.S. Patent No. 6,132, 971) describe the detection of an electronic signal as the means for determining the presence or absence of a target nucleic acid.

✓ 8. Hollis, et al., (U.S. Patent No. 5,891,630) describe the use of electrical signals for the detection of target analytes bound to a solid surface.

Based on the above examples, and numerous others not cited, applicants submit that electronic detection methods for the detection of analytes are well known in the art.

Accordingly, the present invention is directed toward techniques that can be used to increase the signal, decrease the noise or make it easier to detect the signal over background noise. Specifically, the present invention applies methods of processing electronic output signals in biological systems.

The Examiner's Rejections under 35 U.S.C. § 112, first paragraph

In rejecting claims 1-9 under 35 U.S.C. § 112, first paragraph, the Examiner outlines the factors cited Ex Parte Forman to support his position that the specification is not enabling and that undue experimentation would be required. These factors are: 1) the quantity of experimentation necessary; 2) the amount of direction or guidance presented; 3) the presence or absence of working examples; 4) the nature of the invention; 5) the state of the prior art; 6) the relative skill of those in the art; 7) the predictability or unpredictability of the art; and 8) the breadth of the claims.

Undue Experimentation is Not Required to Practice the Methods of the Present Invention

With respect to the first factor, the quantity of experimentation necessary to apply methods of processing output signals in electrical systems to biological systems is not undue. As is fully outlined in the specification, well known techniques are used for the electronic detection of target analytes. In addition, these techniques are well known in the art.

With respect to the second factor, applicants submit that the amount of guidance within the specification coupled with knowledge known in the art for electronic detection of

signals is high. Assay methods for the electrochemical detection of target analytes are well known in the art. The specification provides ample guidance for electrochemical detection assays that would benefit from the application of processing output signals to increase the signal to noise ratio. For example, basic detection mechanism are described which rely on the detection of an ETM based on electron transfer through a target analyte (see pages 5-7). Target analytes which may be detected electronically are described (see pages 7-11). A description of various electrodes which when connected to an electronic device, sense a current and convert it to a signal is provided in the specification at pages 11-13. Methods for enhancing electrochemical detection through the addition of self-assembled monolayers are provided in the specification at pages 13-31. Means for capturing a target analyte, attaching ETMs, and amplifying the signal by the addition of extra labels are provided on pages 32-81. A number of systems are described for the electronic detection of target analytes on pages 81-91. Lastly, the application of AC voltametry theory to these systems is described on pages 91-116, with specific emphasis on increasing the signal to noise ratio using methods to make the system more nonlinear (i.e., square wave harmonic ACV) and method for separating the signal from the background (i.e., Fourier Transformation, Joint-Time Frequency Transformation, impedance analysis, digital filtering techniques, etc.). Therefore there is ample guidance for applying signal processing techniques to the electrochemical detection of target analytes. Therefore there is ample guidance for applying signal processing techniques to the electrochemical detection of target analytes.

As to the third factor, the presence or absence of working examples, supports enablement. As noted by the Examiner, working examples are provided in the specification. Applicants note that a specific working example for each embodiment is not required as long as the specification, combined with the prior art provide sufficient guidance to one of skill in the art *(See, e.g., Vas-Cath Inc. v. Mahurkar*, supra).

As to the fourth factor, the nature of the invention, supports enablement. The nature of the claimed invention is directed to methods of detecting target analytes in a sample. As outlined above, assay methods for the electrochemical detection of target analytes are well known in the are and sufficient guidance is provided in the specification.

The fifth factor, the state of the prior art, also supports a finding of enablement. As outlined above, several patents and publications have been published describing methods for detecting target analytes using electrochemical detection.

The sixth factor, the relative skill of those in the art, also leads to a finding of enablement. As noted by the Examiner, the relative skill of those in the art of which the invention most closely pertains is high.

As regards the eighth factor, the breadth of the claims, this analysis also supports a finding of the enablement. The claims as drafted are commensurate with the scope of the invention.

Regarding the seventh factor, the level of unpredictability associated with the exact properties of any particular variant does not negate the other factors. The Examiner's focus on this one factor, to the exclusion of the other seven, is not proper under the Federal Circuit's analysis.

Accordingly, the applicants submit that the specification coupled with the state of the art fully enables the skilled artisan to make and use the claimed invention without undue experimentation and request that the rejection under 35 U.S.C. § 12, first paragraph be withdrawn.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

Specifically, the Examiner states in claims 1 and 10, it is unclear as to what kind of signal will be defined as a "first input signal". New claims 11-13 recite "electronic input signals" for technical clarity, and thus the rejection should be withdrawn.

Additionally, the Examiner states in claim 2 that the term "higher harmonic analysis" is a relative term which is not defined in the specification. New claim 16 clarifies that the analysis comprises the analysis of higher harmonic signals. Furthermore, Applicants submit that the term "higher harmonic signals" is a term of art in the electronic field.. That is, in the field of electronics, harmonic refers to a periodic wave or a quantity have a frequency that is an integral multiple of the fundamental frequency which can be separated into components in which each component has frequency that is an integral multiple of the fundamental frequency. This concept is demonstrated in Figure 3. Figure 3 shows the 2d harmonic, the 4th harmonic, and the 6th harmonic components of the harmonic signals comprising a square wave ACV. Accordingly, applicants respectfully request withdrawal of the rejection.

Rejection Under 35 U.S.C. § 102(a)

Claim 1 is rejected under 35 U.S.C. § 102(a) as being anticipated by Livache, *et al.*, (1998) Anal. Biochem., 225:188-194.

As stated above, the present invention is directed to the use of signal processing methods for the electrochemical detection of biomolecules.

Livache, *et al.*, teach methods for detecting specific sequences of nucleic acids using fluorescence microscopy. Specifically, Livache, *et al.* teach methods in which detection is achieved through the electrosynthesis of a conducting polymer film. Briefly, DNA chips are prepared by covering electrodes with a conducting polymer to which oligonucleotide probes are attached. Following hybridization of a biotinylated amplified sample, detection is carried out by fluorescence microscopy through an R-phycoerythrin label. Thus, Livache, *et al.*, teach methods for the fluorescent detection of nucleic acids.

New claims 11-13 recite electronic input signals; as Livache et al. does not teach or suggest electronic signals, the rejection should be withdrawn.

The Commissioner is authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1300 (our Order No. A-65686-1/RFT/RMS/RMK).

Dated: 3/26/01

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The first paragraph beginning on page 122, has been amended as follows:

– Monolayer Deposition on Circuit Boards

As above, the circuit boards were removed from the foil-lined bags and immersed in a 10% sulfuric acid solution for 30 seconds. Following the sulfuric acid treatment, the boards were immersed in two Milli-Q water baths for 1 minute each. The boards were then dried under a stream of nitrogen. The boards were placed on a X-Y table in a humidity chamber and a 30 nanoliter drop of DNA deposition solution was placed on each of the 14 electrodes. The DNA deposition solution consisted of 33 uM thiolated DNA, 33 uM 2-unit phenylacetylene wire (H6), and 16 uM undec-1-en-11yltri(ethylene glycol)($HS-CH_2)_{11}-(OCH_2CH_2)_3-OH$) in 6x SSC (900 mM sodium chloride, 90 mM sodium Citrate, pH 7) w/1% Triethylamine. 3 electrodes were spotted with a solution containing DNA 1 (SEQ ID NO:1) (5'-ACCATG GACACAGAT$(CH_2)_{16}$SH-3'). 4 electrodes were spotted with a solution containing DNA 2 (SEQ ID NO: 2) (5'TCATTGATGGTCTCTTTTAACA($(CH_2)_{16}$SH-3'). 4 electrodes were spotted with DNA 3 (SEQ ID NO: 3) (5'CACAGTGGGGGGACATCAAGCAGCCATGCAAA$(CH_2)_{16}$SH-3'). 3 electrodes were spotted with DNA 4 (SEQ ID NO: 4) (5'-TGTGCAGTTGACGTGGAT$(CH_2)_{16}$SH-3'). The deposition solution was allowed to incubate at room temperature for 5 minutes and then the drop was removed by rinsing in a Milli-Q water bath. The boards were immersed in a 45°C bath of M44 in acetonitrile. After 30 minutes, the boards were removed and immersed in an acetonitrile bath for 30 seconds followed by a milli-Q water bath for 30 seconds. The boards were dried under a stream of nitrogen and stored in foiled-lined bags flushed with nitrogen until use.. –

The second paragraph begining on page 122, has been amended as follows:

– Hybridization and Measurement

The modified boards were removed from the foil-lined bags and fitted with an injection molded sample chamber (cartridge). The chamber was adhered to the board using double-sided sticky tape and had a total volume of 250 microliters. A hybridization solution was prepared. The solution contains 10 nM DNA target (SEQ ID NO:5) (5'-TGTGCAGTTGACGTGGATTGTTAAAAGAGACCAT CAATGAGGAAGCTGCAGAATGGGATAGAGTCATCCAGT-3' (D-998), 30 nM signaling probe (D-1055) and (SEQ ID NO: 6) 10 nm 5'-TCTACAG(N6)C(N6)ATCTGTG

TCCATGGT-3' (N6 is shown in Figure 1D of PCTUS99/01705; it comprises a ferrocene connected by a 4 carbon chain to the 2' oxygen of the ribose of a nucleoside). The signalling probe is as follows:

(SEQ ID NO: 7) 5'-$(C23)_4$-N87-N87-N87-N87-ATC CAC GTC AAC TGC ACA-3' (D- 1055)

|	|	|	|
C23	C23	C23	C23
C23	C23	C23	C23
C23	C23	C23	C23
C23	C23	C23	C23

N87 is a branch point comprising a ring structure. C23 is shown in Figure 1F of PCTUS99/01705.

In a solution containing 25% Qiagen lysis buffer AL, 455 mM $NaClO_4$, 195 mM NaCl, 1.0 mM mercaptohexanol and 10% fetal calf serum. 250 microliters of hybrid solution was injected into the cartridge and allowed to hybridize for 12 hours. After 12 hours, the hybridized chip was plugged into a homemade transconductance amplifier with switching circuitry. The transconductance amplifier was equipped with summing circuitry that combines a DC ramp from the computer DAQ card and an AC sine wave from the lock-in amplifier (SR830 Stanford Instruments). Each electrode was scanned sequentially and the data was saved and manipulated using a homemade program designed using Labview (National Instruments). The chip was scanned at between -100 mV and 500 mV (pseudo Ag/Ag/Cl reference electrode) DC with a 25 mV (50 mV peak to peak), 1000 Hz superimposed sine wave. The output current was fed into the lock-in amplifier and the 1000 Hz signal was recorded (ACV technique). The data for each set of pads was compiled and averaged.

	Ip	Relative Intensity Ip
DNA 1 (SEQ ID NO: 1) (Positive 2 Fc)	34 nA	0.11
DNA 2 (SEQ ID NO: 2) (Positive Sandwich Assay)	218 nA	0.7
DNA 3 (SEQ ID NO: 3) (Negative)	0.3 nA	0.001
DNA 4 (SEQ ID NO: 2) (Positive Sandwich Assay)	317 nA	1

On page 128, immediately preceding the claims, the Sequence Listing was added to the text.